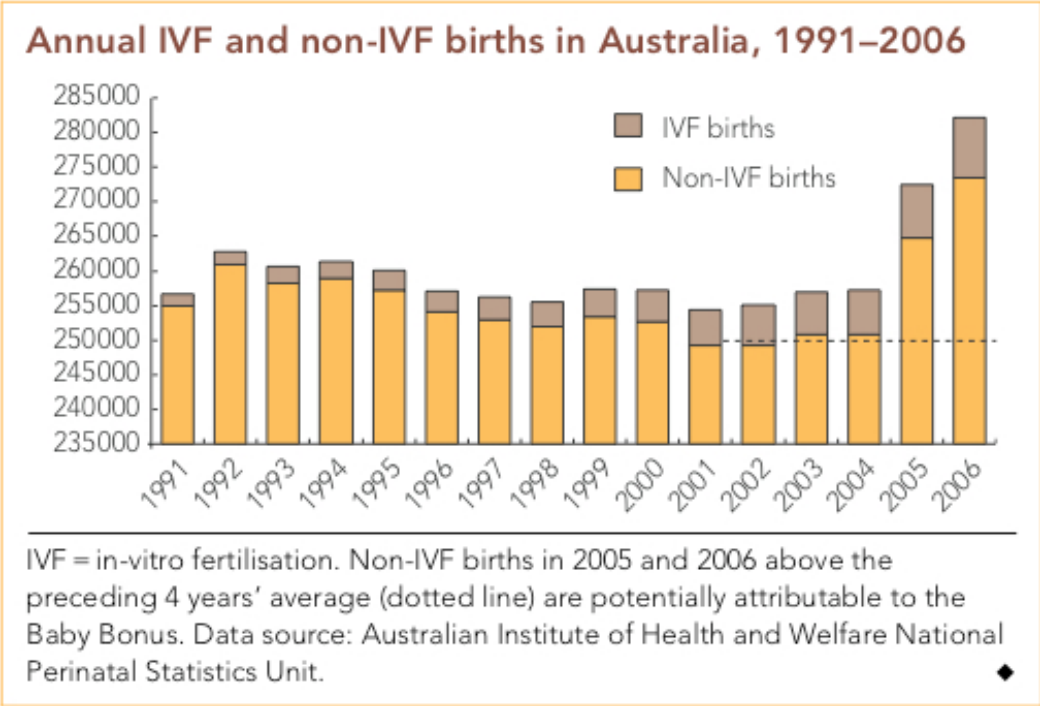
ASSISTED REPRODUCTIVE TECHNOLOGY

By Tim Chang **September 2009**

1-3% all live births are from ART
there is an increasing proportion as well as absolute number of births conceived by IVF



from R Jansen S Dill editorial MJA 2009

Reasons:

- Improved success rates
- Increasing incidence infertility esp older women
- Improved access including cost.

Issues in the management of infertility

1. patient age
2. cause of infertility
3. duration
4. prior treatment
5. cost of treatment

need to differentiate:

- Absolute sterility eg
  ovarian failure/anovulation
  blocked tubes
  azoospermia
  needs assisted conception
- Relative subfertility.
  in which IVF will accelerate conception

Indications of ART

tubal disease

a) distal tubal disease conception success depends on the extent of damage

After tubal surgery	mild	70% conception
	moderate	30% conception → consider ART if ≥ 35
	severe	0-15% → consider ART

b) proximal tubal disease - success <5% - 60% depending on extent of disease and experience

consider IVF if the LBR after 1 cycle of IVF > cumulative LBR after 2 years post surgery
NB TB → sterility

Endometriosis (see endometriosis notes)

- mild
  70% conception rate within 12 months with expectant treatment (retrospective studies).
  1 RCT showed ablation/excision resulted in increase PR with fecundity of 5% vs 2%
- moderate
  with adhesions 30% PR → can be increased with surgery 40 –50%
- severe
  0-10% conception without therapy
  25-30% conceive with surgery and most do so within 18/12 post surgery

endometriosis →

- decreased response to Gntp stimulation
- decreased oocyte quality
- decreased fertilization rates
- possible reduced implantation rates

but modern IVF techniques of down regulation with COH overcome most of these factors and there is no difference PR/cycle with IVF in patients with endometriosis independent of stage compared to other causes infertility.
Recent studies 20-40% PR/cycle

Male factor

ICSI has revolutionised treatment male factor infertility.
Empirically %rapid motile sperm x % normal forms x sperm count > $1x10^6$ for chance natural (Perssons personal communication 2003) conception
Ideally for successful IUI

total sperm count (TW)	$>5x10^6$
motility	>50%
morpholgy (strict)	>1%

Unexplained infertility

Natural conception depends on:

- age
- duration of infertility

Management:

Treatment for unexplained infertility	fecundity
expectant	1-3
clomid	4-6
Clomid + IUI	7-9
Gntp + IUI	9-16
IVF + ET	20-50

Ovulatory dysfunction
Treatments:

- clomiphene
- gonadotrophins
- pulsatile GnRH
- IVF
- Donor eggs

Other causes
Immunological eg antisperm antibodies
Uterine causes

Investigations prior to IVF

- D3 FSH/E2
- Antithyroid abs and TFTs
- US ± SSG
- Trial wash ASA and SCSA
- Androgen profile
- Antenatal screening (including hepB/C/HIV male+female)
- genetic screening eg CF, thalassaemia

Selected Cases

- Karyotype
- Laparoscopy

General advice for IVF

- Stop smoking (PR RR 0.54)
- Reduce alcohol
- Optimize weight → improved PR
- Folic acid

Prognostic factors for success of IVF

1)Age

Age is the most important prognostic factor for natural fertility and determining success of therapy

age	Relative natural pregnancy rate
<35	1
35-40	0.9
40-44	0.62
>45	0.14

Female fecundity declines with age especially after 38-40 years

Decline in fertility due to:

(i) decreased oocyte availability secondary to the failing gonad

(ii) impaired implantation secondary - abnormal hormonal environment
- defective oocyte/embryo

(iii) increased chromosomal abnormality and abortion

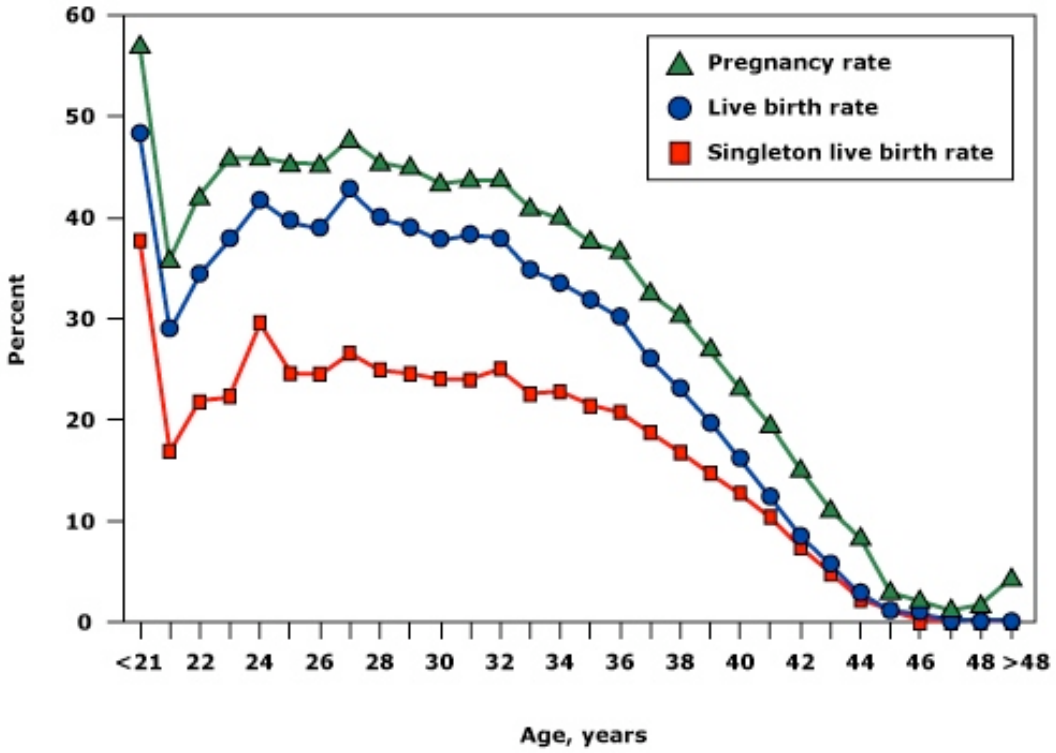
(iv) increased medical and gynaecological diseases e.g endometriosis / fibroids / DM

fertility decline in IVF patients precedes natural fertility by 2 years i.e. women ≥ 33 undergoing IVF have declining success rates

few live birth with ART in women ≥ 44 (using their own eggs)

few pregnancy with ART in women ≥ 45 (using their own eggs)

Pregnancy rates, live birth rates, and singleton live birth rates for ART cycles using fresh nondonor eggs or embryos, by age of woman,* 2005



A woman's age is the most important factor affecting the chances of a live birth when her own eggs are used. The above figure shows the percentages of pregnancies, live births, and singleton live births for women of different ages who had ART procedures using fresh nondonor eggs or embryos in 2005. The percentages of ART cycles resulting in live births and singleton live births are different because of the high percentage of multiple-infant deliveries counted among the total live births. The percentage of multiple-infant births is particularly high among women younger than 35. Among women in their 20s, the percentages of ART cycles resulting in pregnancies, live births, and singleton live births were relatively stable; however, success rates declined steadily from the mid-30s onward.

2) Ovarian reserve

- FSH < 20 and ideally <15

if D2 FSH > 20 repeat FSH / E2 over 3 days to confirm or clomiphene challenge
FSH is a moderate predictor poor ovarian response but low predictor of nonpregnancy

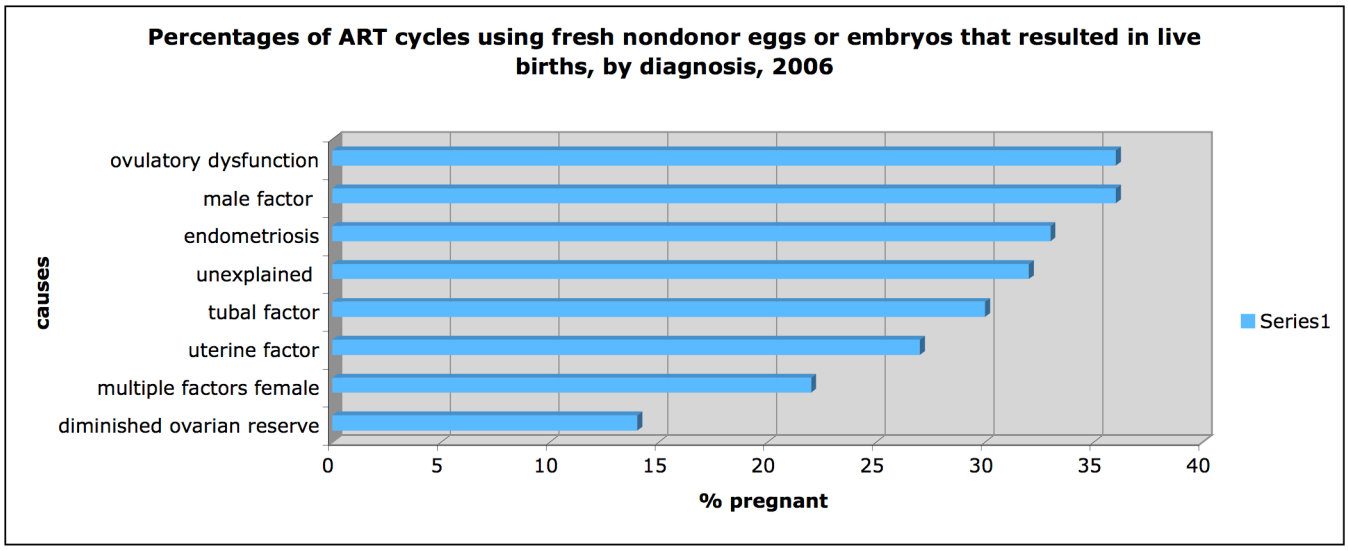
- E2

High E2 associated with rapid premature follicle recruitment and reduced oocyte numbers → poor pregnancy outcome in IVF.
Liccardi etal. Fert Ster 1995 in observational study 592 IVF cycles without GnRHa found no PR with E2 >275pmol/L.

- AFC / ovarian volume measurements
- AMH
- inhibinB

3) Cause of infertility

Ovulatory dysfunction / male factor better than unexplained better than diminished ovarian reserve.
Untreated hydrosalpinx → reduced PR



This figure shows the percentage of ART cycles that resulted in live births according to the causes of infertility. Although the national average success rate was about 29 percent, success rates varied somewhat depending on the couple's diagnosis; however, the definitions of these diagnoses may vary from clinic to clinic. In general, couples diagnosed with tubal factor, ovulatory dysfunction, endometriosis, male factor, or unexplained infertility had success rates above the national average. The lowest success rate was observed for those with diminished ovarian reserve. Additionally, couples with uterine factor, "other" causes, or multiple infertility factors had below-average success rates. Please note, however, that a review of select clinical records revealed that reporting of infertility causes may be incomplete. Therefore, differences in success rates by causes of infertility should be interpreted with caution. Reproduced from 2006 Assisted Reproductive Technology Success Rates, National Summary and Fertility Clinic Reports, Centers for Disease Control, published 2008.

4) number and quality of embryos to choose from for ET

greater number and quality of choice of embryos, the higher the PR

Steps in IVF

1) superovulation
2) oocyte pickup (OPU)
3) in vitro fertilisation and embryo culture
4) embryo transfer (ET)

Ovarian stimulation for IVF

Aim of controlled ovarian stimulation is to obtain multiple high quality mature oocytes for successful fertilization and subsequent pregnancy and minimizing the risks of OHSS.

Conventional Gntp stimulation →

- unpredictable onset and intensity of ovarian response
- premature LH surge (10-20% cycles) → ↓ oocyte quality/fertilization rates / PR

hence use of GnRHa down regulation protocols.

Combined GnRHa downregulation (lucrin 0.5-1mg sc daily) with Gntp:
Benefits:

- ↓ premature LH surge and cancelled cycles
- OPU more predictable
- ↑ PR / oocytes

disadvantages

- luteal phase insufficiency (LH) hence needs luteal phase support
- ↑OHSS
- expensive

most protocols use recombinant FSH:

- consistency with no LH contamination
- unlimited production
- low immunogecity allowing SC administration
- reduced infectious risk
- improved potency???

clinical challenges in COH

- young patients (overly sensitive to Gntp)
- older patients (difficult to stimulate)
- PCOS (unpredictable response)
- Obese patients (require higher doses Gntp)
- Absent LH (threshold LH levels required for stimulation)

Protocols LH t ½ = <60min FSH t ½ = 4-36 hours HCG t ½ = 48 - 96 hours

a) short
GnRHa D1 to ovulation trigger
Gntp D2 to HCG

b) ultrashort
GnRHa D1-5 (minimum 5 days to reliably inhibit LH surge not to occur within 7 days)
Gntp D2 to HCG
Significant ↓ PR with pure FSH secondary to profound ↓ LH after ceasing GnRHa

c) long protocol ***Most COH in IVF use long protocols***
GnRHa mid luteal phase previous cycle for min 7 days to suppress endogenous Gntp and marked by E2<200pmol/L and continued until HCG
Risk of follicular cysts formation with GnRHa → ↑E2
Mx follicular cysts:
- continue GnRHa upto 21 days. (>21 days GnRHa may → slight ↑ cancellation rates)
- TV cyst aspiration
- Cancel cycle

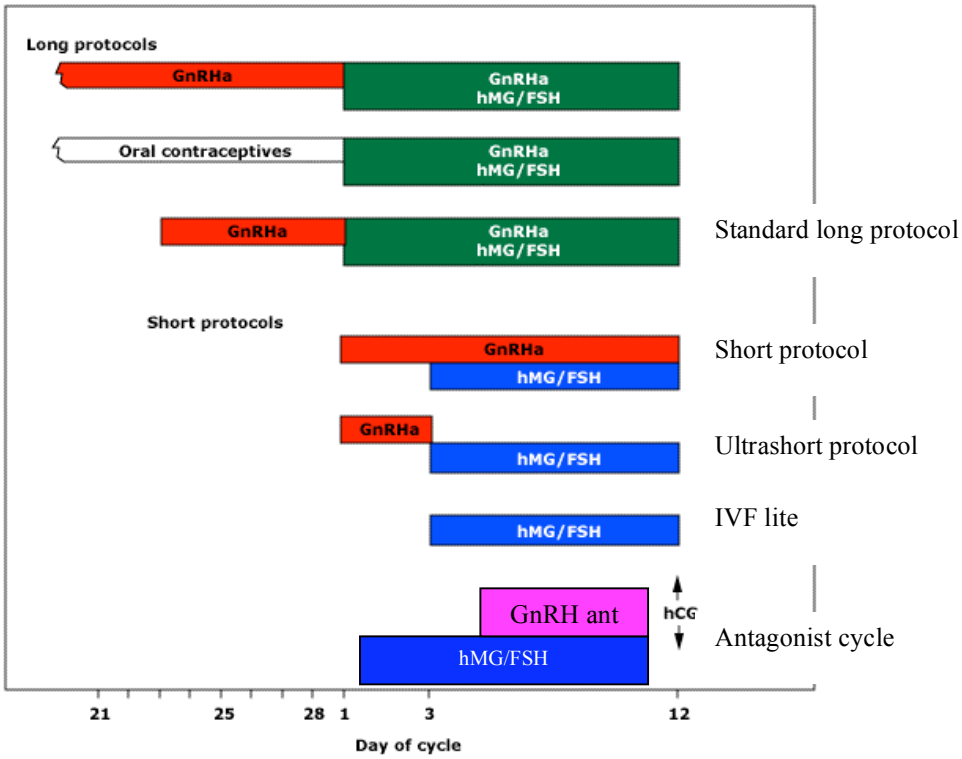
Adding provera D 19- 23 or OCP for >14 days will suppress any flare follicular/luteal cysts
Advantages OCP suppression:
- prevents functional cysts
- prevents inadvertent pregnancy
- convenience
- improves response Gntp with ↑ follicles from improved synchronization of cohort of follicles

Once pituitary suppression documented commence Gntp (usually D2 of menses)
(+) improved follicular development →↑fertilization rates → ↑ PR
(-) ↑ Gntp dose
↑ OHSS

d) IVF lite
No GnRHa used
Gntp D3 until trigger
Aim is to secure ≈ 4 follicles
(+) Maybe useful for poor responders
reduced OHSS
(-) Risk of spontaneous LH surge

e) GnRH antagonists (available 8/2001)
GnRH antagonist commenced after starting Gntp once follicle >13mm ± E2 >1000 (variable regmen)
Monitor LH and E2 / follicle response as antagonist can → profound drop in LH → ↓PR
Single dose 3mg suppress LH surge upto 96 hours
Daily dose 0.25mg daily cetrotide until HCG trigger
advantages:
- more convenient, shorter stimulation and monitoring
- ↓ OHSS (RR 0.61) especially PCOS
- reduced overall cost cycle with ↓monitoring/duration of therapy

disadvantages:
- decreased PR (RR 0.82) (Cochrane review 2006) secondary to profound drop LH secretion (LH needed for production of androgens which are aromatized to estrogens for follicular development) Use of GnRH antagonists in many studies suboptimal and there is suggestion with optimal use GnRHant PR similar with long protocols



Criteria for triggering:

- ≥ 1 follicle > 16-18mm (ideally > 20mm)
- ≥ 2 additional follicles 15-16 mm
- E2 > 500pmol/L per follicle >15mm
- Endometrial thickness > 8mm (type 2 or 3)

Trigger 3000 – 10 000IU HCG or 250iu recombinant HCG and time OPU 35-37 hours after injection
Do not perform OPU <34 hours post trigger as oocyte may not be mature
IVF lite OPU 34-35 hours in case spontaneous LH surge missed
For those at risk of OHSS:

- GnRHa to trigger in antagonist cycle
- Recombinant LH may be used to trigger as ↓ t ½ may → ↓ OHSS

need to institute luteal support early as LH surge may not support implantation

Luteal Phase Support (LPS)

Ovarian stimulation with supraphysiological levels steroids / GnRHa → impairs LH secretion required to support corpus luteum (in most stimulated IVF cycles) which produces progesterone, relaxin integrins etc to prepare the endometrium for implantation. Hence need luteal phase support
HCG RR 2.72 Progesterone RR 1.57 :

1) HCG 1500IU D+4 and +8 after trigger
   NB *HCG may → F(+) pregnancy test if performed too early within 96 hours of last HCG*
2) Progesterone pessaries 200-600mg daily or Crinone progesterone gel (90mg)
   Some evidence in long cycles E2 supplementation(E2 valerate 6mg) + prog may improve PR

Table 1: Summary of studies evaluating the addition of E_2 to progesterone on LPS in different stimulation schemes

COH	Long GnRH agonist				GnRH antagonist
	Smitz *et al.* (1993)	Fahri *et al.* (2000)	Lukaszuk *et al.* (2005)	Lewin *et al.* (1994)	Fatemi *et al.* (2006
Number of patients	378	271	231	100	201
Dose of E_2 (mg)	6	2	6, 2 and 0	2	4
Implantation rate, % (Progesterone with E_2 versus progesterone)	32.8 versus 35.5	15.2* versus 10.2	29.9* versus 17.8 versus 9.8	_	42.4 versus 37.8
Clinical PR/ET, % (Progesterone with E_2 versus progesterone)	29.2 versus 29.5	39.5* versus 25.6	51.3* versus 32.8 versus 23.1	28 versus 26.5	32.6 versus 28.9

*$P < 0.05$. COH, controlled ovarian hyperstimulation.

from from Human Reproduction Update, Vol.13, No.6 pp. 581–590, 2007

3) IM progesterone 25-100mg daily. (meta analysis 2002 IM better than PV progesterone for PR and delivery rate RR 1.33) but significant SE preclude 1st line use:
    - Pain
    - Abscess formation
    - Eoisnophilic peumonia

Some evidence improved PR with HCG support as other hormones beside progesterone needed to prepare the endometrium.
If there is increased risk factors for OHSS then use progesterone

Table 2: Comparison of the main differences of two meta-analysis using different LPS schemes

	HCG versus Progesterone		
	HCG versus vaginal progesterone (RR) Pritts and Atwood (2002)	HCG versus IM progesterone (RR) Pritts and Atwood (2002)	HCG versus Progesterone (vaginal and IM) (OR) Nosarka et al. (2005)
Number of patients	707	486	438
Clinical PR/ET	0.9 (95% CI 0.72–1.14)	0.98 (95% CI 0.68–1.42)	1.71 (95% CI 1.06–2.76)
Delivery rate	–	1.7 (95% CI 0.52–6.27)	–

RR, relative risk; OR, odds ratio.

meta analysis HCG vs progesterone for LPS
from Human Reproduction Update, Vol.13, No.6 pp. 581–590, 2007

Grading risk for OHSS:

- Low risk no monitoring required
  E2 < 6000
  Low oocyte count

- Medium risk measure E2 / progesterone luteal phase and use progesterone pessaries as required
  E2 6-10,000
- High risk
  E2 > 10,000
  > 15 follicles
  treatment:
    Freeze all
    Monitoring with progesterone pessaries
    Cancel cycle and OPU

Cycle cancellation

- Medical
    - Delayed follicular growth / poor stimulation
    - Premature LH surge
    - High risk OHSS eg. ≥ 20 follicles esp high number <15mm / E2 > 25000
- Non medical reasons

Oocyte Pick Up (OPU)

Most OPU performed by TV US

Advantages:

- Simple, low cost and easy to learn
- Low complication rate
- No hospitalization / OT required

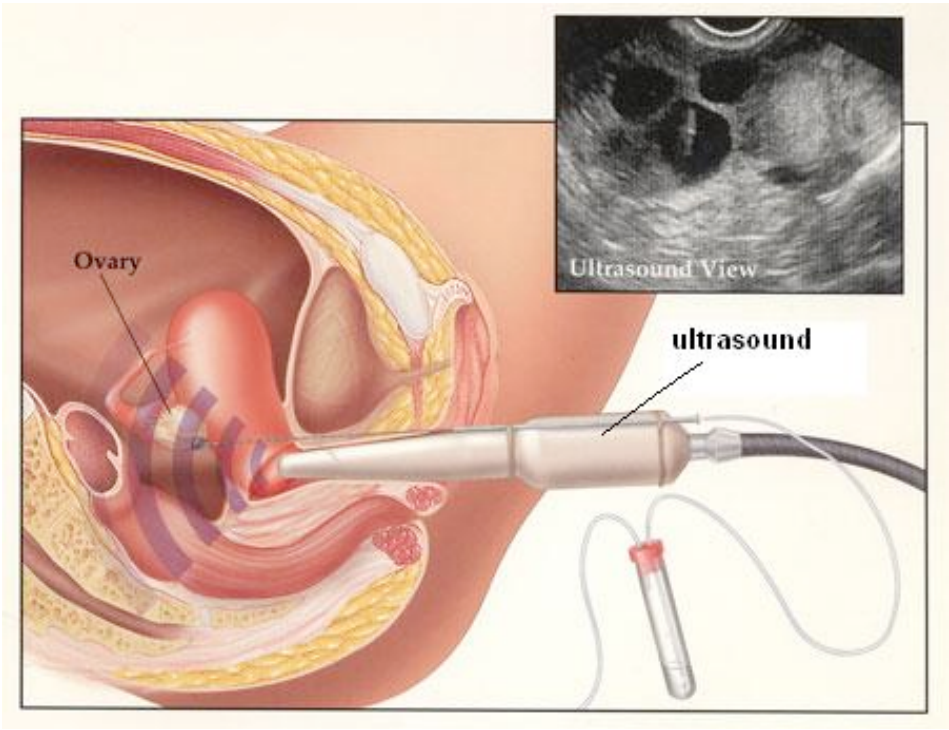
Risk: (<1/1000)

- Vascular injury
- Infection
- Bowel injury

Timing of OPU 35-37 hours post HCG trigger.

If no GnRHa used, time OPU 34 hours post trigger lest spontaneous LH surge.

Waiting >37 hours → fragile post mature or post ovulatory follicles



In Vitro Fertilisation

3 steps:

- Oocyte maturartion
- Sperm preparation
- Insemination and fertilization

Oocyte preparation

Oocytes matured in-vitro after aspiration for 4-6 hours post OPU and graded before insemination. (invitro maturation 2 –24 hours depending on maturity of oocyte and ideally inseminate 2-6 hours after 1st polar body extruded)

Oocyte graded on maturity:

- Grade 1 – immature prophase 1
- Grade 2 – near mature metaphase 1
- Grade 3 – mature metaphase 2
- Grade 4 – post mature

IVF media commercially available and may contain:

- Serum → improved culture
- Co culture systems i.e. additional heterologous cells to aid growth of embryos especially to blastocyst

sperm preparation

aims:

- Remove seminal plasma which prevents capacitation and fertilization of oocytes
- Concentrate motile sperm
- Remove detritus and contaminants

Various techniques of centrifugation to prepare sperm e.g.

- Percoll 40/80
- Swim up technique
- Glass wool filtration

Require $5x10^6$ motile normal morphology sperm for IVF

Insemination and fertilization

Each well with oocytes placed with $0.1x10^6$ motile normal morphology sperm and cultured for 16-20 hours until pro-nuclei stage (2PN) and assessed. Any abnormal number of PN (1-25%) should be discarded as these embryos may be indistinguishable after fusion.

Steps in fertilization:

1) sperm capacitation (glycoprotein of sperm removed to allow fertilization)
2) acrosome reaction → sperm acquire perforation in acrosome which allow penetration of zona pellucida (ZP)
3) fusion of sperm and oocyte membranes →
    - release of sperm chromosomes into oocyte cytoplasm → formation male pronulceus
    - oocyte completes meiosis from metaphase 2 → extrusion 2nd polar body and formation of female pronulceus
    - release of corticol granules from oocyte cytoplasm which blocks polyspermy

Fusion of PN 16-24 hours post insemination → single cell zygote → undergoes cleavage 2cells (24 hours) → 4 cells (44hours) → 8 cells (>48hours post insemination) → morula → blastocyst

Embryo Transfer

ET can occur anywhere between 2PN to blastocyst
Conventionally ET 48-72 hours post fertilization
SIVF routinely transfer at D5

Factors to consider in ET

1) number of embryos to TF
2) type of embryos to TF
3) technique of ET

Number of embryos to transfer

Aim of ART is to produce a livebirth with minimal morbidity, hence success is not only to achieve pregnancy, but to prevent multiple and high order multiple pregnancies which have significantly more morbidity and mortality and higher costs.

Increase SET from 2002 28% to 57% in 2006 (Wang MJA 2009) with resultant reduction twin births form 19% to 11%

Most IVF units in Australia 2009 in good prognosis patients do eSET 1st IVF cycle

Cochrane review 2009 concluded:

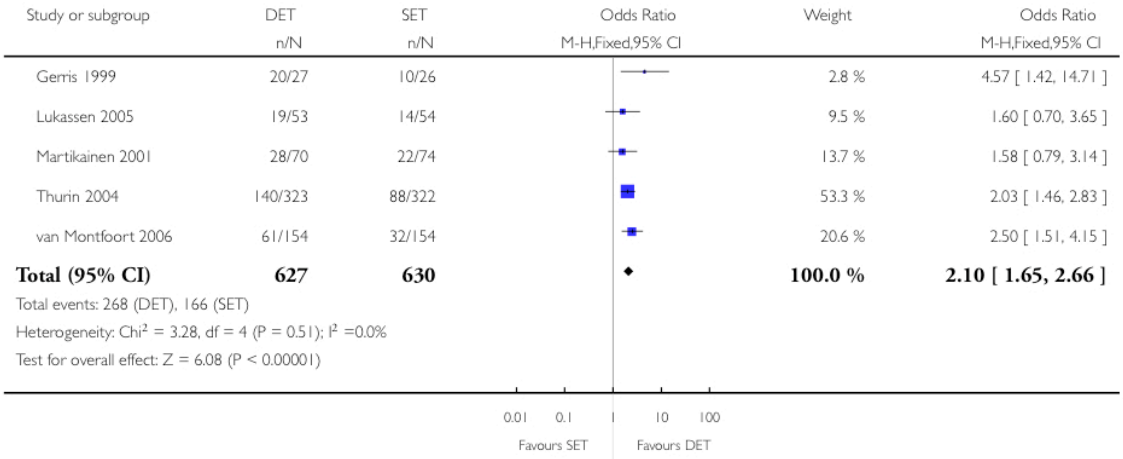
- DET leads to higher LBR vs SET (RR 2.1) but

Analysis 1.1. Comparison 1 DET versus SET, Outcome 1 Livebirth rate.

Review: Number of embryos for transfer following in-vitro fertilisation or intra-cytoplasmic sperm injection

Comparison: 1 DET versus SET

Outcome: 1 Livebirth rate



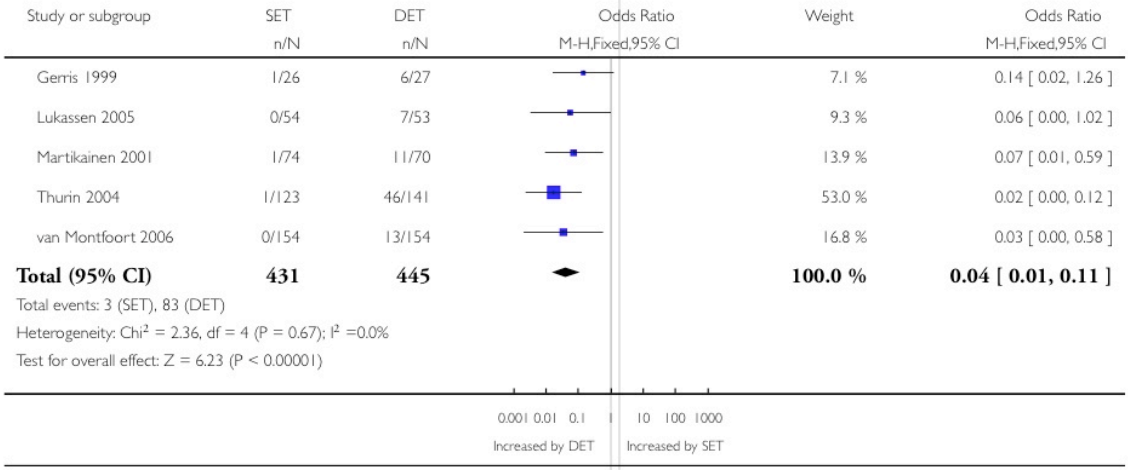
- SET leads to significantly less MPR (RR 0.04)

Analysis 1.3. Comparison 1 DET versus SET, Outcome 3 Multiple pregnancy rate.

Review: Number of embryos for transfer following in-vitro fertilisation or intra-cytoplasmic sperm injection

Comparison: 1 DET versus SET

Outcome: 3 Multiple pregnancy rate



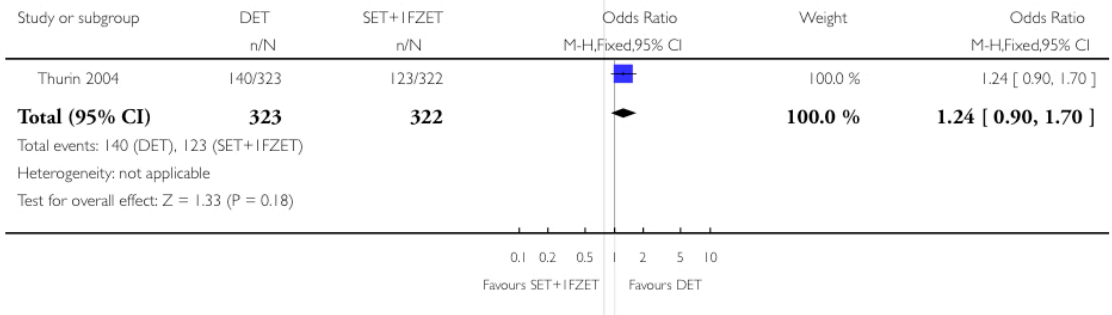
- There NSD in LBR between DET and SET + subsequent FET

Analysis 2.1. Comparison 2 DET versus SET plus1FZET, Outcome 1 Livebirth rate.

Review: Number of embryos for transfer following in-vitro fertilisation or intra-cytoplasmic sperm injection

Comparison: 2 DET versus SET plus1FZET

Outcome: 1 Livebirth rate



Methods to improve pregnancy rate in ART cycles

1) Blastocyst D5 transfer

Using sequential media:

Zygote – D3 → pyruvate

D4+ → glucose utilization

IR 50-80% with high grade embryos

Best implantation with:

Grading of blastocyst

Formation of blastocyst at D5 vs D7

Embryonic genome is not activated until 4-8 cell stage (48-72 hours post fertilization) hence D3 embryos may not reflect true viability(euploidy) of embryo:

- 1/3 high grade D3 embryos followed to blastocyst were not the best quality when cultured to D5.
- 60% high grade D3 embryos aneuploid vs 35% D5(Papanikolaou NEJM 2006)

RCT women <36 in their first 2 IVF cycle good prognosis patients delivery rate higher D5 vs D3 (RR1.48)

Table 2. Rates of Pregnancy and Delivery after the Transfer of Single Blastocyst-Stage and Cleavage-Stage Embryos.

Variable	Single Blastocyst-Stage Embryo Transferred (N=175)	Single Cleavage-Stage Embryo Transferred (N=176)	Relative Risk (95% CI)*	P Value
	% (no.)			
Rate/patient randomly assigned to treatment				
Pregnancy†	41.7 (73)	33.5 (59)	1.23 (0.95–1.63)	0.11
Clinical pregnancy	33.1 (58)	23.3 (41)	1.42 (1.01–2.00)	0.04
Ongoing pregnancy	33.1 (58)	21.6 (38)	1.54 (1.08–2.18)	0.02
Pregnancy loss‡				
Ectopic pregnancy	1.4 (1)	1.7 (1)		
1st Trimester	19.2 (14)	33.9 (20)	0.57 (0.31–1.02)	0.07
2nd Trimester	2.7 (2)§	0		
Delivery	32.0 (56)	21.6 (38)	1.48 (1.04–2.11)	0.03
Multiple births	0	5 (2)	0.14 (0.01–2.77)	0.16
Rate/patient starting stimulation¶				
Pregnancy	43.2 (73)	34.5 (59)	1.25 (0.96–1.64)	0.10
Clinical pregnancy	34.3 (58)	24.0 (41)	1.43 (1.02–2.01)	0.04
Ongoing pregnancy	34.3 (58)	22.2 (38)	1.54 (1.09–2.19)	0.01
Delivery	33.1 (56)	22.2 (38)	1.49 (1.05–2.12)	0.03
Rate/embryo transfer‖				
Pregnancy	48.7 (73)	37.6 (59)	1.30 (1.00–1.68)	0.05
Clinical pregnancy	38.7 (58)	26.1 (41)	1.48 (1.06–2.06)	0.02
Implantation**	38.7 (58)	27.4 (43)	1.41 (1.02–1.95)	0.04
Ongoing pregnancy	38.7 (58)	24.2 (38)	1.60 (1.13–2.25)	0.007
Delivery	37.3 (56)	24.2 (38)	1.54 (1.09–2.18)	0.01

* CI denotes confidence interval.
† Pregnancy was defined by a positive human chorionic gonadotropin test.
‡ The percentages are based on 73 initial pregnancies in the blastocyst-stage group and 59 initial pregnancies in the cleavage-stage group.
§ One patient underwent elective termination of pregnancy at 15 weeks for anencephaly of the embryo, and one patient had a second-trimester miscarriage at 13 weeks.
¶ The percentages are based on 169 patients in the blastocyst-stage group and 171 patients in the cleavage-stage group.
‖ The percentages are based on 150 patients in the blastocyst-stage group and 157 patients in the cleavage-stage group.
** The implantation rate is the number of gestational sacs with a fetal heartbeat divided by the number of embryos transferred.

from Papanikolaou NEJM 2006

SIVF <38 with fresh ET

	PR	IR	Twin PR
2x D2-3 embryos	40%	30%	35%
Single D5 embryo	40-45%	40%	1-2%
2x D5 embryo	50%	40%	40+%

Advantages blastocyst TF

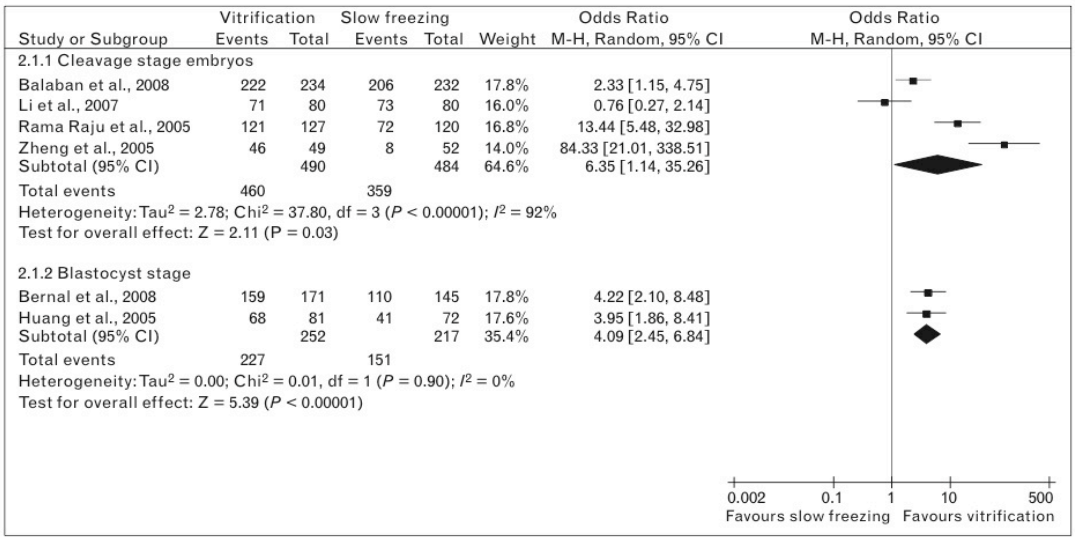
- High IR
  40-80% IR due to best quality embryos will reach blastocyst and be selected
- Reduced multiple PR
  2xD5 ET 40% twin PR → 1% twin PR with single D5 ET
- PGD more options available

Disadvantages

- Failure to achieve blastocyst
  In poor responders no embryos may reach blastocyst
- Monozygotic twinning ↑ 5%
- Increase size offspring

2) Scoring of embryo quality
   from oocyte → PN → cleavage embryo → blastocyst quality can be graded
   - Morphology (grading 1-3 Dokras or Gardiner's grading 1-6/1A-C/A-C)
   - time to reach various stages there is an optimal time e.g.
     4 cells at 48 hours
     blastocyst at D5
   - metabolic markers
3) assisted hatching
   limited evidence women >38, laser hatcing ZP may improve IR
4) vitrification of frozen embryos
   FET → ↓ IR and PR vs fresh ET ? related selection process ? freezing process?
   Use of virtification (rapid freeze over minutes) improved LBR by 50% vs slow freeze (SIVF 2006)
   70-90% survive thawing process.
   Rapid thaw techniques → ↑ PR
   congenital abnormalities and adverse obstetric outcome need further study
   Meta analysis 2008 vitrification vs slow freeze:
   - improved post thaw survival embryos (D3 RR 6.35 ; D5 RR 4.09)

Figure 1 Odds ratios for postthawing survival rates of embryos cryopreserved by vitrification or slow freezing at the cleavage or blastocyst stage



CI, confidence interval.

- NSD in PR or LBR (numbers are small)

5) others e.g.
   - metabolic profile of embryos → animal studies successful embryos have ↑ glucose uptake
   - PGD / PGS to assess genetic and chromosomal competence of embryo
     Indications PGD:
     - Known familial genetic disease
     - Multiple miscarriages
     - Multiple failed IVF cycles with appropriate morphology embryos
     - Age?

Factors in ET affecting PR

1) gentle atraumatic technique (SIVF use soft bulb transfer catheter)
2) ET under TA US from RCT → ↑ PR 50%
3) Full bladder may → easier ET
4) Site embryo deposition
   Miduterus deposit → ↓ EP versus fundal deposition.
   Use of US to assess cervical length and depth of insertion more accurate than blind insertion.
5) dummy / mock ET prior to real ET → ↑PR

Factors that do not affect ET success rates

bed rest
slow catheter withdrawal
antibiotics ((+) cultures ↓ PR but AB do not improve this)
experience of the clinician (once properly trained)

Frozen Embryo Transfer (FET)

Preparation of the endometrium:

- natural cycle
- programmed cycle → ↑ PR
  more convenient as can manipulate timing of ET

SIVF protocol

Artificial follicular phase 12-20 days
Ethinyl estradiol suppresses endogenous E2 production, therefore measured serum E2 reflects placental production after ET

D1-7 30μg EE
D8-14 50μg EE D10: US endometrium >6mm type 2 or 3
E2 <200pmol/L (ensures ovarian suppression)
D 15-30 20μg EE + 100mg progesterone suppositories BD
Aim midluteal progesterone >20-25 pmol/L
PV progesterone better absorbed than PO or IM
D20 blastocyst TF
D21 progesterone assay
D 30 BhCG if no menses

If pregnant:

- Continue EE until E2 > 200pmol/L
- Continue progesterone until independent placental production progesterone (up to 12 weeks)

vitrification of embryos → improved PR compared to slow freeze

Complications of IVF

1) unsuccessful conception
2) ovarian hyperstimulation syndrome (OHSS)
3) complications OPU eg pelvic infection / haemorrhage / laparotomy
4) ovarian torsion
5) multiple pregnancy
6) ?ovarian cancer?

Results and types of ART

Standard IVF with fresh ET

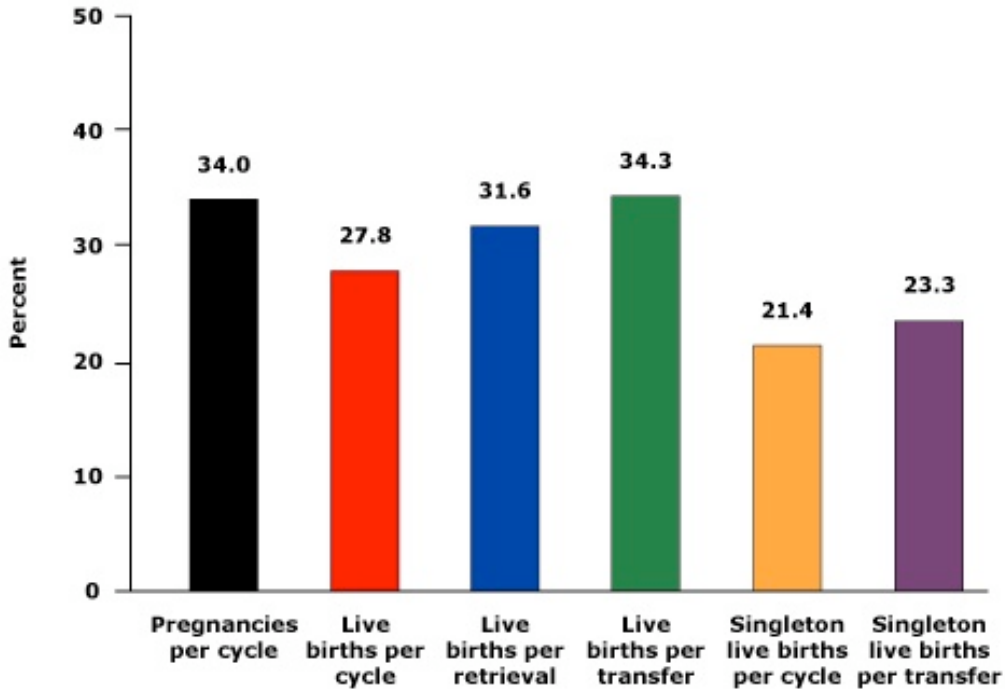
pre-requisite:

minimal number of sperm is required ≈ 10^6 active normal sperm
normal uterus and cervix present
oocytes

be aware how units define success:

- numerator = biochemical PR / (+) FHB / live birth
- denominator = per ET / per OPU / per cycle started etc

Success rates for ART cycles using fresh nondonor eggs or embryos, by different measures, 2005



The above figure shows ART success rates using six different measures, each providing slightly different information about this complex process. The vast majority of success rates have increased slightly each year since CDC began monitoring them in 1995.

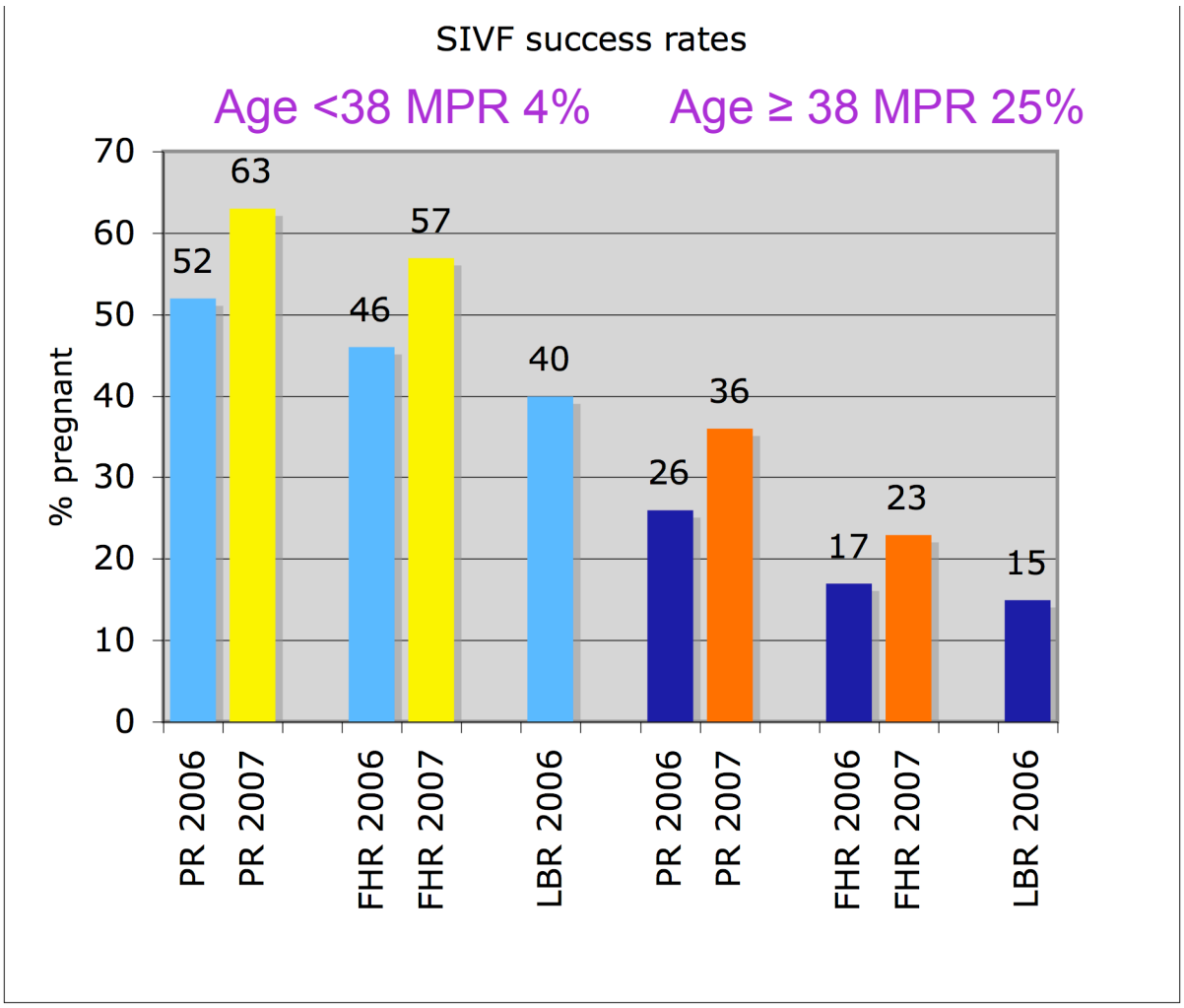
Percentage of ART cycles started that produced a pregnancy: this is higher than the percentage of cycles that resulted in a live birth because some pregnancies end in miscarriage, induced abortion, or stillbirth.

Percentage of ART cycles started that resulted in a live birth (a delivery of one or more live-born infants): this is the one many people are most interested in because it represents the average chance of having a live-born infant by using ART. This is referred to as the basic live birth rate in the Fertility Clinic Success Rate and Certification Act of 1992.

Percentage of ART cycles in which eggs were retrieved that resulted in a live birth: this is generally higher than the percentage of cycles that resulted in a live birth because it excludes cycles that were canceled before eggs were retrieved. In 2005, about 12 percent of all cycles using fresh nondonor eggs or embryos were canceled for a variety of reasons. This is referred to as the live birth rate per successful oocyte (egg) retrieval in the Fertility Clinic Success Rate and Certification Act of 1992.

Percentage of ART cycles in which an embryo or egg and sperm transfer occurred that resulted in a live birth: this is the highest of these six measures of ART success.

Percentage of ART cycles started that resulted in a singleton live birth: overall, singleton live births have a much lower risk than multiple-infant births for adverse infant health outcomes, including prematurity, low birth weight, disability, and death.



Percentage of ART cycles in which an embryo or egg and sperm transfer occurred that resulted in a singleton live birth: this is higher than the percentage of ART cycles started that resulted in a singleton live birth because not all ART cycles proceed to embryo transfer. Reproduced from: Centers for Disease Control and Prevention, American Society for Reproductive Medicine, Society for Assisted Reproductive Technology. 2005 Assisted Reproductive Technology Success Rates: National Summary and Fertility Clinic Reports, Atlanta: Centers for Disease Control and Prevention, 2007.

❖ PR dependent age mainly

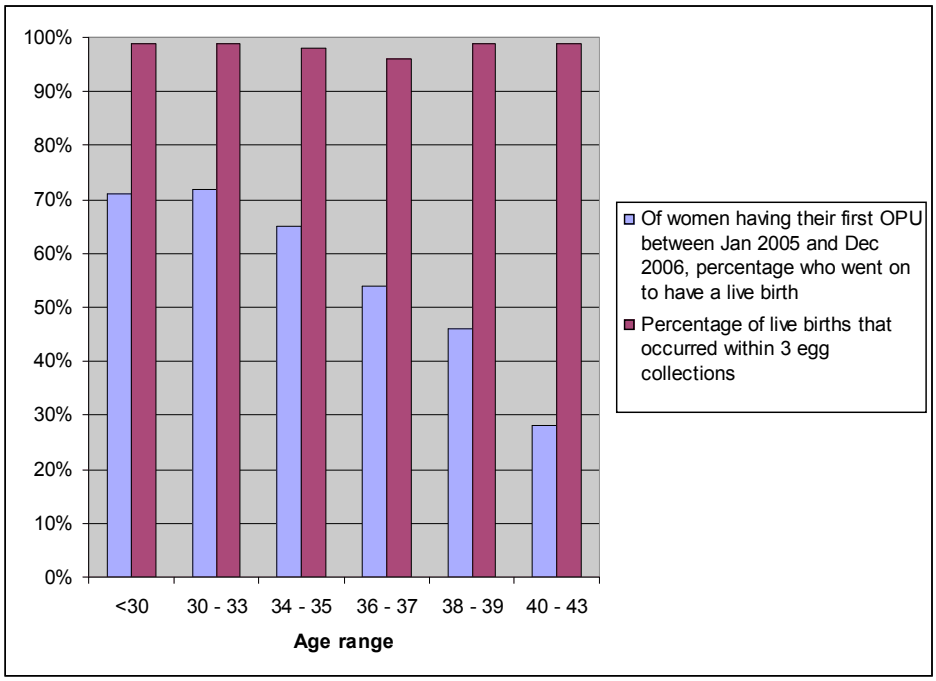
Age	PR / fresh ET	PR / FET	Live birth rate
<35	45%	28%	35%
35-40	30%	18%	25%
>40	10%	7%	6%
overall	30%	20%	25%

overall pregnancy rate per OPU for 1st attenders SIVF 45%
overall pregnancy rate per OPU for <38 at SIVF >60%

SIVF success rates

age	Number of ET	PR per ET 2006	LB rate per ET 2006
<25	36	58	52
25-34	1238	45	41
35-37	771	36	31
38-39	458	28	24
>39	709	17	13

most women destined to conceive at SIVF, majority do so within 3 stimulated cycle



Cumulative PR per OPU SIVF 1998 n=648

Age	PR	Ectopic PR	MC	Twins	LBR (combined)	LBR (FET)	IR
<35	62%	2%	10.5%	23%	52%	34%	25%
35-39	37.5%	1%	16%	20%	30%	33%	13%
40-44	21%	0	43%	6%	12%	25%	7%
total	44%	1.4%	15.7%	20.5%	35%	33%	

❖ multiple pregnancy rates

SET has reduced the twin PR rate from 40% DET to 4% SET

1 Number (%) of babies born to women following SET or DET procedures, Australia and New Zealand, 2002–2006

	SET	DET	Total
Singletons	13 468 (96.0%)	16 100 (60.9%)	29 568 (73.0%)
Multiples	554 (4.0%)	10 361 (39.1%)	10 915 (27.0%)
Twins	532 (3.8%)	10 090 (38.1%)	10 622 (26.3%)
Triplets or higher	22 (0.2%)	271 (1.0%)	293 (0.7%)

DET = double embryo transfer. SET = single embryo transfer.

from Wang et al. MJA 2009. 190:234

Cochrane review DET vs elective SET:

- DET → higher LBR versus SET with significantly higher twin PR
- Cumulative PR fresh and frozen ET between SET and DET NSD

❖ ectopic pregnancy 4% in patients with tubes

expected lower EP rates with modern IVF as:

- more aggressive removal of damaged fallopian tubes
- single ET

❖ Miscarriage

Minimal effects of modern IVF on miscarriage rates

(SIVF 1998 MC rate 12% overall and increased with age)

MC rate increases with age

ICSI
for male infertility
intracytoplasmic injection (ICSI) has made other techniques male infertility obsolete.
Requires viable sperm DNA for fertilization →SCSA testing pre IVF
ICSI has not improved PR in non male factor infertility
70%+ fertilisation rate
30 – 40%+ pregnancy rate/TF

Frozen embryo transfer
10-15 % lower PR / TF compared with fresh TF related mainly to poorer quality embryos are frozen and the best ones are transferred fresh.

allows female to have multiple embryo T/F per stimulated cycle (OPU)

meta analysis 2009 fresh vs frozen ET:

- No increase adverse obstetric outcome
- Most studies showed no increase in congenital abnormalities (except Belva et al 2008: frozen ICSI embryos ↑ abnormalities RR 2.15)

Donor oocytes
Indications:

- advanced ovarian age
- poor responders
- previous multiple failed IVF attempts
- ovarian failure

much improved implantation rates due to

(i) lack of stimulated cycle recipient
(ii) better quality oocyte for donor

GIFT
GIFT is obsolete with modern IVF ET success rates without risk of laparoscopy

Cancer with ovulation induction/IVF

Beware of pitfalls of studies:

- Small numbers
- Short followup
- Confounding factors for cancer risk e.g. infertility; anovulation; endometriosis etc see what controls are used
- Retrospective studies → recall bias

Ovarian cancer
Early studies suggest ↑ ovarian cancer/ borderline tumours RR 2-11 with OI however most controls are general population.
More recent studies confounding for nulligravity, infertility, anovulation etc do NOT show increase risk

Breast cancer
Biologically plausible ↑ E2 and progesterone → breast cancer however:
Conflicting evidence if Gntp → increase breast cancer and some studies show clomiphene → decrease risk

Endometrial cancer
Concern OI → increase risk e.g tamoxifen, however most studies have methodological flaws and there is no good evidence

Complications of ART pregnancy versus normal pregnancy

1) multiple pregnancy
   rate of 20-50% with multiple ET.
   with elective single blastocyst ET, rates of multiple pregnancies are reduced and should match normal population
   policy SIVF to transfer 1 embryo in women <38 years undergoing 1st cycle IVF.
2) Ectopic pregnancy
   rate of 5% of all ART, but this is decreasing with modern IVF techniques
   increased heterotopic pregnancy rate related to >1 ET
   natural conception 1:30,000
   ART up to 1:100
3) Miscarriage
   dependent on female age
   Related to multiple pregnancy

4) congenital abnormality
   increased major abnormality RR = 2 (Hansen et al)

	% congenital abnormal
IVF	9
ICSI	8.6
Normal population	4

   Meta-analysis Hansen 2005
   RR all birth defects 1.3-1.4 (baseline risk 2-4%)
   RR major birth defects 2
   Number IVF/ART births to have 1 extra congenital abnormality 62-250 births
   Unknown if related to infertility per se or IVF process or recall bias in studies.
   Increased CP (RR = 1.7) up to 14 years in case control study (Stromberg at al)
5) preterm birth rate for singleton IVF conceptions
   RR 2.04 <37weeks
   RR 3.27 <32weeks
   singleton SET PTB is lower than singleton DET
   frozen ET lower PTB than fresh ET

5 Perinatal outcomes of babies born following SET or DET procedures, Australia and New Zealand, 2002–2006

	SET	DET	OR (95% CI)	AOR* (95% CI)
All babies				
Low birthweight of liveborn babies	8.7%	24.8%	3.44 (3.23–3.68)	3.55 (3.32–3.80)
Preterm birth of all babies	12.3%	30.3%	3.10 (2.92–3.28)	3.19 (3.01–3.38)
Fetal death of all babies†	9.9%	14.4%	1.46 (1.20–1.77)	1.49 (1.21–1.82)
Singletons				
Low birthweight of liveborn singletons	6.6%	7.9%	1.21 (1.11–1.32)	1.15 (1.05–1.26)
Preterm birth of all singletons	10.1%	11.3%	1.14 (1.06–1.23)	1.13 (1.05–1.22)
Fetal death of all singletons†	8.6%	10.9%	1.27 (1.00–1.60)	1.26 (0.98–1.62)
Multiples				
Low birthweight of liveborn multiples	61.4%	51.2%	0.66 (0.55–0.79)	0.60 (0.50–0.72)
Preterm birth of all multiples	67.1%	59.8%	0.72 (0.60–0.87)	0.66 (0.55–0.80)
Fetal death of all multiples†	41.5%	19.9%	0.47 (0.30–0.73)	0.46 (0.29–0.73)

AOR = adjusted odds ratio. DET = double embryo transfer. OR = odds ratio. SET = single embryo transfer.
* Adjusted for women's age, cause of infertility, parity, number of previous assisted reproductive technology treatments, type of embryo, and stage of embryo development. † Number of fetal deaths per 1000 births. ◆

from Wang et al. MJA 2009. 190:234

2 Perinatal outcomes of babies born following SET or DET procedures, by type of embryo, Australia and New Zealand, 2002–2006

Number and type of embryo	Total liveborn babies	Proportion of LBW liveborn babies	P*	Total babies	Proportion of preterm births (all babies)	P*	Fetal deaths/ 1000 births (all babies)	P*
SET								
Fresh embryo	9121	9.5%	< 0.01	9229	12.9%	< 0.01	10.1	0.79
Thawed embryo	4736	7.3%		4793	11.2%		9.6	
DET								
Fresh embryo	18402	27.7%	< 0.01	18737	32.5%	< 0.01	16.1	< 0.01
Thawed embryo	7620	17.7%		7724	25.0%		10.4	
All								
Fresh embryo	27523	21.7%	< 0.01	27966	26.0%	< 0.01	14.1	< 0.01
Thawed embryo	12356	13.7%		12517	19.7%		10.1	

DET = double embryo transfer. LBW = low birthweight. SET = single embryo transfer. * χ^2 test, df = 1. ◆

from Wang et al. MJA 2009. 190:234

6) Low Birth Weight

increased LBW may be related to drugs rather gametes

	RR		LBW
LBW	2.6	Singleton ART	10%
VLBW	1.3	Non ART	5%

SET singletons have reduced LBW vs DET singletons

Frozen ET have reduced LBW vs fresh

7) Caesarean section rates RR 1.54

	Age <30	30-40	>40
ART	20%	40%	60%+
Non ART	20%	25%	35%

8) Perinatal mortality increased RR 1.68

Effects of repeated IVF cycles on outcome

In general no adverse effects of repeated IVF cycles on various outcomes, except effects of advanced maternal age.

Effects of repeated COH on ovarian response as per maternal age, with no reduction (up to 6 cycles) **Note** cohort of failed IVF patients may be different to those that fall pregnant quickly

No evidence repeated COH → ↓ ovarian reserve

Min decline in fecundability with repeat IVF cycles up to 4 cycles and any decline related female age.

Slight ↑ PR 2nd cycle ? related to adjustment clinical management after 1st cycle

New developments IVF

Egg freezing

- Medical
- Social reasons

Current data:

- woman ≤ 35 need 15 usable eegs to produce 1 pregnancy
- woman ≥ 41 need 40 usable eggs to produce 1 pregnancy. (Molloy MJA 2009)

Combined Genetic Hybridization (CGH)

CGH can analyse all 23 pairs of chromosomes vs FISH analysis upto 7 pairs

Potential in PGS to improve implanatation rates

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